MYCOBIOTA OF SOILS FROM WHEAT GROWING AREAS OF THE CSONGRÁD-TIMIS REGION

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ABSTRACT

Cereals can be attacked by several fungal pathogens, some of which may cause serious yield and quality losses. Besides, several fungal plant pathogens also contaminate cereals with various mycotoxins which are harmful to animals or humans. Several plant pathogenic fungi are soil-borne pathogens. We examined the mycobiota of soil collected in wheat growing areas in the Csongrád-Timis region to get insight into the role of soil in the occurrence of wheat diseases. Soil samples were collected from 10-10 fields in both countries at three different times. Samples were diluted and plated onto appropriate media to identify the fungi. Fungal identifications were carried out using morphological and molecular sequence-based methods. Based on the results, the soil samples contained large amounts of *Trichoderma* isolates. Besides, several potential mycotoxin producers have been identified, including black *Aspergillus* species which are potentially able to produce ochratoxins and fumonisins, *Aspergillus allicaeus*, which is able to produce ochratoxins, several *Penicillium* species which can produce a range of mycotoxins, and several *Fusarium* species including the well-known cereal pathogens and mycotoxin producers *F. graminearum, F. oxysporum* and *F. sporotrichioides*. Among the cereal pathogens, *Pyrenophora teres* and *Cochliobolus lunatus* were also identified in the soil samples. We also identified *Clonostachys rosea* (teleomorph: *Bionectria ochroleuca*) in some of the soil samples. This species produces a range of enzymes which can be used for the biodegradation of zearalenone. This is the first report on its occurrence in Hungary.

Keywords: soil, wheat, sequence-based identification, mycobiota, *Aspergillus, Penicillium, Fusarium*

INTRODUCTION

Agricultural soils are natural, living bodies, which change in space and time. The soils from the cross border region between Hungary and Romania play a major role in the long term sustainability of agriculture in the region. Soil is an important source of plant pathogens of cereals. Fungal pathogens of cereals can either be seedborne (e.g. *Ustilago* sp.), airborne or soilborne. However, most of the fungal pathogens of cereals are able to infect the plant from the soil (or plant debris present in the soil). The various fungal pathogens can cause serious yield and quality losses. Besides, several of these pathogens (e.g. *Fusarium*, *Penicillium* and *Aspergillus* species) also contaminate the cereals with various mycotoxins, secondary metabolites which cause various disease symptoms in animals and humans. The soil is also an important source of fungi which can be used in biocontrol strategies to combat plant diseases, or to lower the mycotoxin content of agricultural products. Prime examples of these useful fungi are *Trichoderma* species which can be used as biocontrol agents against various plant pathogens (KREDICS et al., 2003), or other fungal species which are important sources of mycotoxin degrading enzymes (VARGA and TÓTH, 2005).

In this study, we examined the mycobiota of soil in wheat growing fields in the Csongrád-Timis region to get insight into the role of soil in the occurrence of wheat diseases, and to identify potential candidates which could be used in biocontrol experiments to lower mycotoxin contamination of cereals.

MATERIAL AND METHOD

Collection of soil samples

Soil samples will be collected 3 times, in April, August and November, 2011 in 10 Hungarian and 10 Romanian sampling sites. Samples were collected in each site from two different depths. Seven test sites include wheat fields with intensive cultivation, while 1 site was selected from an organic wheat cultivation field in each country. Test sites including a pasture field and a forest were included as controls. Soils were sampled according to the soil sampling guidelines (90/2008. (VII. 18.) Regulation of Ministry of Rulal Development).

Sample analyses

Appropriate dilutions of the samples were plated on selective media (Dichloran Rose Bengal Agar medium, DRBC, KING et al., 1979) and incubated at 25°C for 1-2 weeks. The colonies were transferred to malt extract agar (MEA) plates and purified. The growing colonies were examined using standard macro- and micromorphological methods (SAMSON et al., 2004). DNA extractions from selected isolates were carried out as described previously (SAMSON et al., 2007). Species assignment of the isolates was carried out using sequence analysis of appropriate gene fragments of the isolates. Usually, the ITS region was amplified and sequenced, while for the species assignment of *Aspergillus* and *Penicillium* species part of the calmodulin gene was used as target (SAMSON et al., 2007).

RESULTS

The mycobiota of soil samples collected from wheat fields from 10-10 locations in the Csongrád-Timis region was examined using morphological and molecular methods. The number of fungal colonies isolated from the soil samples was in general much higher in August than in November or in April. Besides, the soil samples collected from the upper layers of soil were usually more contaminated by fungi than the lower layers (*Figure 1*). Several well-known soil-inhabiting fungi were identified. Among *Trichoderma* species, *T. hamatum, T. koningiopsis, T. virens, T. brevicompactum, T. gamsii* and *T. pleuroticola* were identified. The latter species has recently been described as an important pathogen of oyster mushrooms (KOMON-ZELAZOSWSKA et al., 2007). Our data indicate that a possible source of *T. pleuroticola* is the compost used for mushroom cultivation. Among Fusaria, the well-known cereal pathogens *F. graminearum* and *F. sporotrichioides* (*Figure 2*) were identified, which are producers of a variety of mycotoxins including trichothecenes (deoxynivalenol, nivalenol, T2-toxin) and zearalenone. Besides, *F. oxysporum* was also found in some of the samples.

Aspergillus species have also been identified. Among them, black Aspergilli including *A. niger* and *A. awamori* are potential producers of two carcinogenic mycotoxins, ochratoxins and fumonisins, *A. alliaceus* can produce ochratoxins, while *A. terreus* is able to produce a range of mycotoxins including citreoviridin, terrein and tremorgenic mycotoxins (SAMSON et al., 2011a). *Aspergillus calidoustus* (*Figure 2*) has

recently been described by our group (VARGA et al., 2008). This species is an important opportunistic human pathogen, and is a source of useful metabolites including ophiobolins (SAMSON et al., 2011b). To our knowledge, this is the first report on its occurrence in Hungary.

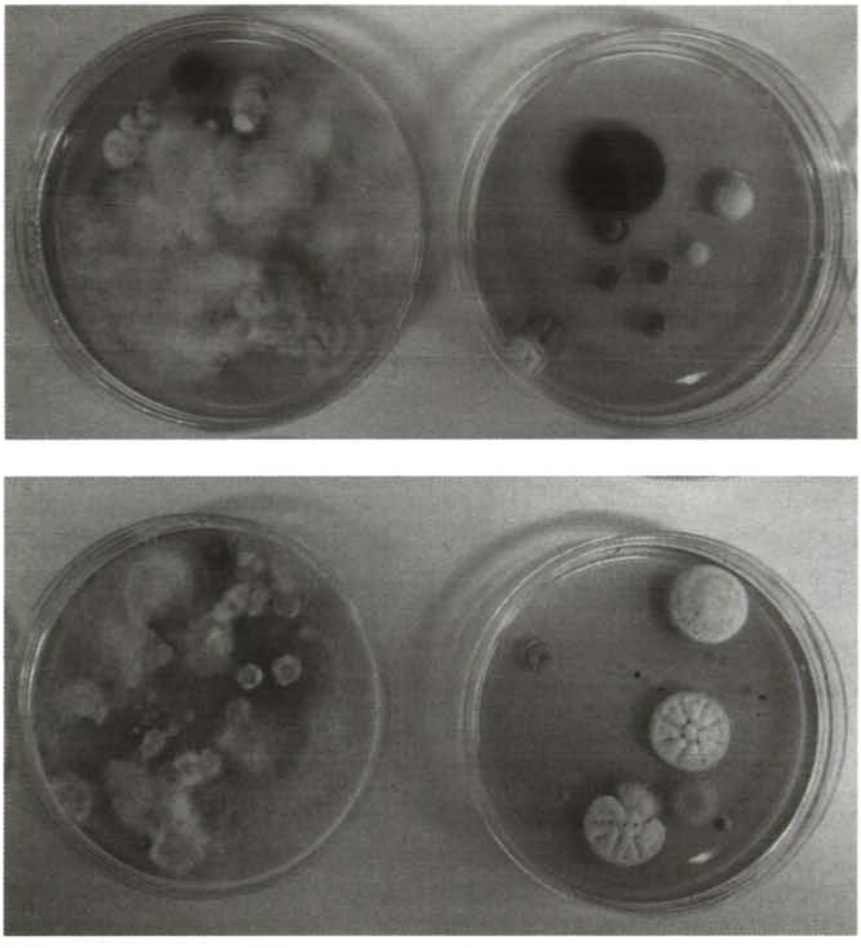

Figure 1: Mycobiota of soil samples taken from the upper (left, 0-15 cm) and the lower (right, 15-30 cm) layers of soil in sampling sites located in Cenad (top) and Sándorfalva (bottom).

In the *Penicillium* genus, most of the isolates were found to belong to the *P. verruculosum* species, which can produce some tremorgenic mycotoxins, and can live as an endophyte in the root system of some plants (BHAGOBATY et al., 2010). Other species identified include *P. glabrum* (*Figure 2*), *P. expansum* (a well-known producer of patulin, a toxigenic compound), *P. griseofulvum*, *P. janthinellum, P. angulare, P. manginii, P. anatolicum* and *P. pinophilum* (recently transferred to *Talaromyces*; SAMSON et al., 2011c). Apart from the mycotoxin producing fungi, well-known plant pathogens including *Pyrenophora teres* and *Cochliobolus lunatus* (synonym: *Curvularia lunata*) have also been identified (*Figure 2*). *Pyrenophora teres* is an important pathogen of cereals especially barley, and it has recently been also been identified on wheat in Hungary (TÓTH et al., 2008).

Another interesting fungus, *Clonostachys rosea* (teleomorph: *Bionectria ochroleuca*) has also been identified for the first time in the soil samples. This species produces a range of enzymes which can be used for the biodegradation of zearalenone (KAKEYA et al., 2002; TAKAHASHI-ANDO et al., 2004).

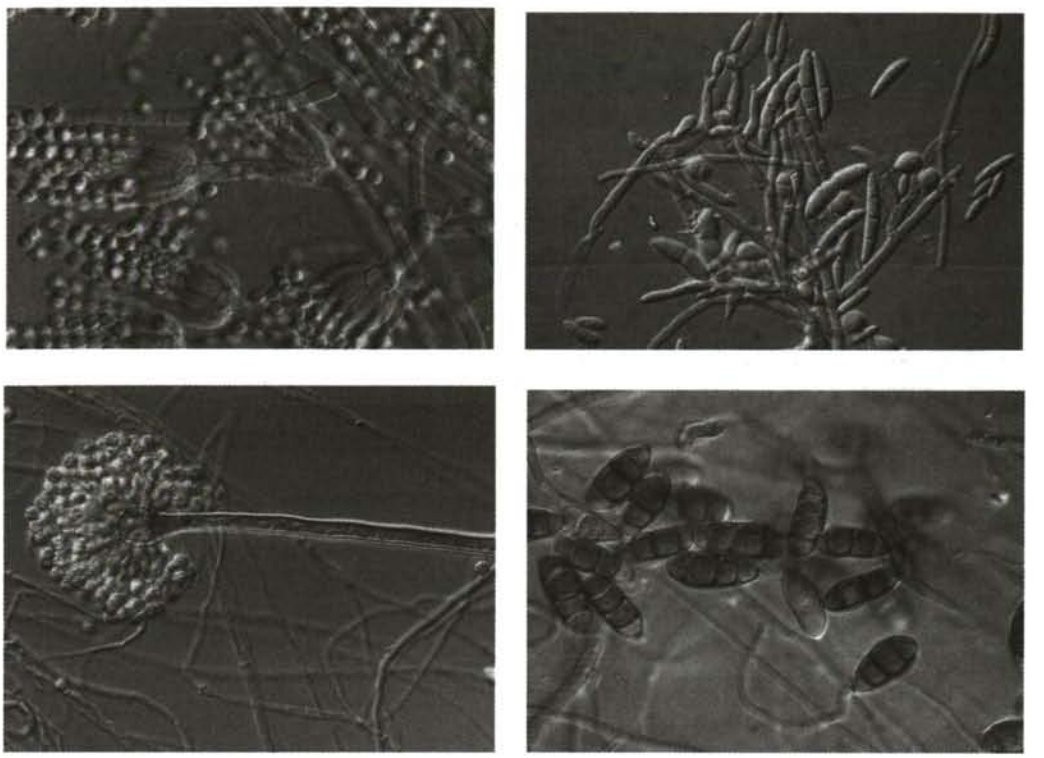

Figure 2: Microscopic pictures of some of the isolates. *Penicillium glabrum* (top left), *Fusarium sporotrichioides* (top right), *Aspergillus calidoustus* (bottom left), *Cochliobolus lunatus* (bottom right).

CONCLUSIONS

Examination of the mycobiota of wheat-growing areas in the Csongrád-Timis region revealed that soil serves as an important source of mycotoxigenic and plant pathogenic fungi in the region. At the same time, soil is also an important and invaluable source of potentially useful fungi which can be used in the biocontrol of plant pathogens, or as sources of enzymes and their genes to be used to detoxify important mycotoxins including zearalenone and others. Further studies are in progress to compare the mycobiota of the soil samples to those of the agricultural products cultivated on these fields.

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